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Relationship between the Adsorption by Carbon Black from Aqueous Solution and the Biopharmaceutical Data of Sulfonamides[1,2]

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As a physico-chemical approach to an understanding of biopharmaceutical phenomena, discussions were given on the basis of the adsorption of sulfonamides by carbon black from aqueous solution.

The hydrophobic interaction was considered to be weakened upon addition of urea, resulting in a decrease in adsorption of sulfonamides from aqueous solution.

The introduction of Me- or MeO- into the molecular structure of sulfonamide caused the increases in the adsorption by carbon black, in the binding to bovine serum albumin, and in the absorption from rat small intestine.

Plotting the adsorption data of sulfonamides against the partition coefficient in butanol-water system reported,[8] a good correlation was given. Then, plotting the same data against the absorption rate from rat small intestine reported,[11] a correlation was observed. It was, therefore, expected that the hydrophobic interaction between the drugs and the intestinal membrane forms an important factor in absorption phenomena.

It was considered in the previous paper[1] that the adsorption of sulfonamides by carbon black might proceed by the hydrophobic interaction between the hydrophobic moiety of the adsorbate molecule and the surface of carbon black.

Based on the consideration that carbon black has a similarity to the biological membrane in the surface chemical aspect because of its hydrophobic property, the correlation between the adsorption by carbon black and the biopharmaceutical data of sulfonamides was discussed as a physico-chemical approach to an understanding of biopharmaceutical phenomena.

Experimental

Materials——Carbon black and sulfonamides used were the same as those in the previous paper.[1] Sulfonamides are abbriviated by the respective sample No., as 1. sulfamethoxypyridazine; 2. sulfadimethoxine; 3. sulfapyridine; 4. sulfamethomidine; 5. sulfamerazine; 6. sulfisomidine; 7. sulfamonomethoxine; 8. 6-sulfanilamido-2-methoxypyridazine; 9. sulfadiazine; 10. sulfaphenazole; 11. slufathiazole; 12. sulfaethidole; 13. sulfamethizole; 14. sulfisomezole; 15. sulfabutamide; 16. sulfanilamide; 17. slufaguanidine; 18. sulfacetamide; 19. N^4-acetylsulfadimethoxine; 20. N^4-acetylsulfisomidine; 21. N^4-acetylsulfamonomethoxine; 22. N^4-acetylsulfisomezole; 23. N^4-acetylsulfadiazine. The rest of the materials were of the purest reagent grade.

Procedure for Determination of the Adsorbed Amount by Batch Method——10 mg of carbon black was added in 10 ml of the respective solutions of sulfonamides in 1/30M Michaelis buffer solution (pH 7.4) or 1/30M Michaelis buffer solution containing urea (pH 7.4), and then the procedure was carried out in the same way as in the previous paper.[1]

Quantitative Determination of Sulfonamides——Concentrations of sulfonamides were determined according to ultraviolet (UV) absorption method in the same way as in the previous paper.[1]

1) This paper forms Part IX of "Physico-chemical Approach to Biopharmaceutical Phenomena." Preceding paper, Part VIII: H. Nogami, T. Nagai, and S. Wada, *Chem. Pharm. Bull.* (Tokyo), **18**, 342 (1970).
2) A part of this work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969.
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Binding of Sulfonamides to Bovine Serum Albumin——Binding constants for sulfonamides with bovine serum albumin were evaluated spectrophotometrically by utilizing the competition with 2-(4′-hydroxyphenylazo) benzoic acid.[4]

Results and Discussion

Demonstration of Hydrophobic Interaction of Sulfonamides with Carbon Black in Aqueous Media

Adsorption of Sulfonamides by Carbon Black from Aqueous Urea Solution——Effect of urea on the hydrophobic interaction has been investigated from biochemical interests.[5] Fig. 1 shows the adsorption isotherms upon addition of urea of the three sulfonamides of different adsorbabilities. The adsorbed amount decreased with the addition of urea. In the previous paper,[6] the effect of urea on the water structure around tryptophan molecule in aqueous solution was discussed from the view that urea comes in contact with the hydrophobic moiety of tryptophan to result in a simultaneous structural change of iceberg around that moiety, accompanying an increase of its affinity to water to result in a predominantly large decrease in enthalpy of mixing. Next, this view was extended to an explanation of the effect of EtOH on the adsorption of tryptophan by carbon black.[6]

The result shown in Fig. 1 also could be explained from the same view. Thus, when urea comes in contact with the sulfonamide molecule (and carbon black surface) to result in a simul-

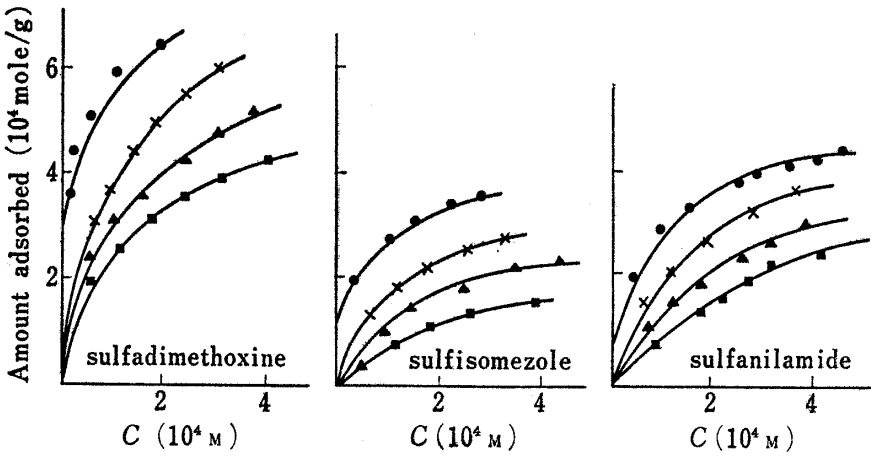


Fig. 1. Adsorption Isotherms of Sulfonamides by Carbon Black from Urea Solution at 40°

C: equilibrium concentration of sulfonamides in Michaelis buffer solution (pH 7.4)
urea concentration: ●: 0M, ×: 1M, ▲: 2M, ■: 3M

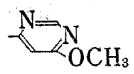
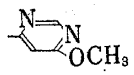
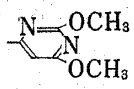
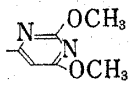
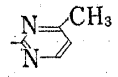
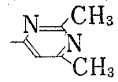
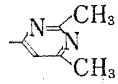
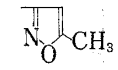
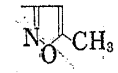
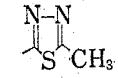
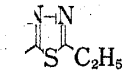
taneous structural change of iceberg around the sulfonamide moleucle (and carbon black surface), the hydrophobicity of sulfonamide (and carbon black) may become smaller than that in the solution without urea, causing a decrease in the escaping tendency of the sulfonamide from solution to carbon black surface to result in a decrease of adsorption.

Thus, the hydrophobic interaction of sulfonamides with carbon black surface was considered to be weakened with the addition of urea.

Increase in Adsorption of Sulfonamides with Introduction of Hydrophobic Groups in the Molecular Structure——There was found no notable correlation between the adsorption of sulfonamides by carbon black and the molcular weight in the previous paper,[1] as was considered

4) I. Moriguchi, S. Wada, and T. Nishizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 601 (1968).
5) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **238**, 4074 (1963).
6) H. Nogami, T. Nagai, and H. Umeyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 328 (1970).

Table I. Relationship between Adsorbability and Biopharmaceutical Data of Sulfonamides

No.	Sulfonamides	R_1[a]	R_2[a]	pK_a	M[b]	$-\Delta F$[c]	log K[d]	K_u[e]
7	sulfamonomethoxine	$-NH_2$		5.96	4.9	5.17	3.3	1.44
21	N^4–acetylsulfamonomethoxine	–NHAc		6.00	5.8	6.13	3.4	—
2	sulfadimethoxine	$-NH_2$		6.32	6.5	6.45	5.5	2.16
19	N^4–acetylsulfadimethoxine	–NHAc		6.01	6.7	6.80	5.5	—
9	sulfadiazine	$-NH_2$		6.52	3.2	5.14	2.9	1.14
23	N^4–acetylsulfadiazine	–NHAc		6.35	4.4	5.47	3.3	—
5	sulfamerazine	$-NH_2$		6.98	5.6	6.10	3.7	1.27
6	sulfisomidine	$-NH_2$		7.57	5.1	5.91	3.1	0.48
20	N^4–acetylsulfisomidine	–NHAc		7.08	6.4	6.36	3.7	—
14	sulfisomezole	$-NH_2$		6.04	2.7	5.16	3.7	—
22	N^4–acetylsulfisomezole	–NHAc		5.54	5.4	5.98	3.8	—
13	sulfamethizole	$-NH_2$		5.20	3.0	5.16	4.3	0.93
12	sulfaethidole	$-NH_2$		5.64	3.5	5.43	4.5	1.33

a) R_1-  -SO_2NH-R_2
b) amount adsorbed at 10^{-4}M equilibrium concentration at 40° (10^4 mole/g)
c) free energy changes by adsorption
d) logarithm of binding constant to bovine serum albumin at pH 7.4 and 37°
e) ref. 11

due to the variety of the molecular structure. Thus, the adsorbabilities of several sulfonamides were compared with those of their substituted compounds, as shown in Table I. The adsorbability and the free energy change of adsorption increased with the introduction of Me^-, MeO^-, or Ac^-, that is, with the increase in hydrophobicity, supporting that the adsorption proceeds by the hydrophobic interaction. This result had a relation to the biopharmaceutical data, as will be described later.

Adsorption of Sulfonamides by Carbon Black from Aqueous Solution in Relation to the Partition Coefficient in Butanol-Water System

The relationships between partition coefficient and biological activity have been discussed regarding various durgs.[7] Fig. 2 shows a good correlation between the adsorbability of sul-

7) For example, B.B. Brodie, H. Kurz, and L.S. Schanker, *J. Pharmacol. Ext. Ther.*, **130**, 20 (1960); C. Hansch, K. Kiehs, and G.L. Lawrence, *J. Am. Chem. Soc.*, **87**, 5770 (1965).

fonamides and the partition coefficient in butanol–water system reported by Rieder.[8] It is, therefore, shown that the hydrophobic interaction plays an important role on the adsorption and there remains a high possibility of the correlation between such an adsorbability and the biological activity.

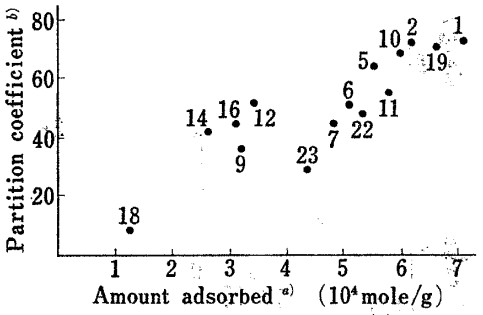


Fig. 2. Relationship between Adsorbability and Partition Coefficient in BuOH–Water System

a) at 10^{-4}M equilibrium concentration at 40°
b) ref. 9

Actually, the above adsorbability comes from a solid–liquid system, while the partition coefficient comes from a liquid–liquid system, and accordingly it is expected that the adsorbability gives another useful information for understanding of biopharmaceutical phenomena, which is not given from a partition coefficient. That is because a biopharmaceutical system probably consists of solid–liquid, for example, the membrane where drugs permeate. Moreover, the adsorbability by carbon black may afford a useful means for an investigation of the biopharmaceutical interaction of another drug or additive which is administered at the same time.

Adsorption of Sulfonamides by Carbon Black from Aqueous Solution in Relation to the Binding to Bovine Serum Albumin

It is known that the binding of drug to such a protein as albumin varies with the origin of protein, *i.e.*, with the animals which produce the samples of albumin.[9] Although the mechanism of protein binding has not been disclosed in detail, one of the most important factors may be formed of the orientation of the bound molecule to the binding site of protein, which is influenced by the conformations of the respective molecules. Therefore, even if albumin of the same origin is used for the experiment, the binding behavior (or binding site) of sulfonamides is considered to vary with the compound because of the variety of the molecular structure. Probably from the above reasons, there was found no notable correlation between the adsorption by carbon black and the binding to bovine serum albumin for all the samples of sulfonamides. Therefore, examinations were made upon the samples of similar molecular structures, as shown in Table I. Both the adsorbability and the binding constant increased with substitution of Me–, MeO–, or Ac–.

pK_a of the compound changes with introduction on Me–, MeO–, or Ac–, but it may not give any distinct effect on the adsorption by carbon black from the solution near the neutral pH region, as was discussed in the previous paper.[1]

Therefore, it is concluded that the increase in hydrophobicity of molecule with the substitution caused the increases in the adsorption by carbon black and in the binding to bovine serum albumin. In other words, the adsorption of sulfonamides by carbon black is considered to correlated to the binding to bovine serum albumin. Additionally, the results demonstrate that a hydrophobic interaction participates in a protein binding, as has been suggested.[10]

Correlation between the Adsorption by Carbon Black from Aqueous Solution and the Intestinal Absorption of Sulfonamides

The absorption rate from rat small intestine, K_u,[11] increases with introduction of Me– or MeO–, *i.e.*, with the increase in hydrophobicity of molecule, as shown in Table I. Plotting the adsorbability by carbon black against the values of K_u of the samples, a correlation was

8) J. Rieder, *Arzneimittel–Forsch.*, **13**, 81 (1963).
9) W. Scholtan, *Arzneimittel–Forsch.*, **11**, 707 (1961).
10) W. Scholtan, *Arzneimittel–Forsch.*, **18**, 505 (1968).
11) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

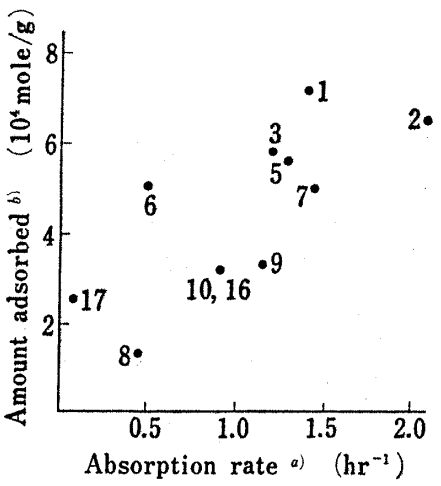


Fig. 3. Relationship between the Intestinal Absorption Data and the Adsorbability of Sulfonamides

a) ref. 11
b) at 10^{-4}M equilibrium concentration at 40°

observed as shown in Fig. 3. The data scattered more aside from a straight line than the plots in the same way of barbituric acid derivatives in the previous paper.[12] This is considered due to the variety of the molecular structure of sulfonamides accompanying the differences among the drugs in various factors such as polar ones.[13]

Conclusively, it is expected that the hydrophobic interaction between the drug and the intestinal membrane forms an important factor in absorption phenomena. However, such a sulfonamide as sulfaguanidine is not considered to follow this generalization, as its intestinal absorption is not good compared with its adsorbability and practically it is administered for enteric disinfection.

Thus further investigations should be made to give the entire picture of drug absorption.

In the case of barbituric acid derivatives,[12] a fairly good correlation was observed between the adsorption by carbon black and the existing pharmacological data. However, there was found no notable correlation between the adsorption by carbon black and the antiseptic activity of sulfonamides. One of the reasons for this result is that the mechanism of drug action of sulfonamides is different from such pharmacodynamic drugs as barbituric acid derivatives because of such factors as polar ones[13], and another reason to be considered is as follows. Considering that both the absorption through membrane and the binding to protein take place on the process of the appearance of drug action of sulfonamides, the increase in hydrophobicity of molecule may cause an increase in the protein binding and a resultant increase in antiseptic activity, but if the binding is too strong to be separated, the activity of drug will decrease, as is well known.[14,15] Therefore, the increase in hydrophobicity of molecule is necessary for the increase in intestinal absorption, but is not always necessary for the increase in antiseptic activity. Accordingly a correlation can not be expected between the adsorbability by carbon black and the antiseptic activity.

12) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **17**, 176 (1969).
13) I. Moriguchi, S. Wada, and T. Nishizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 601 (1968).
14) A.H. Anton, *J. Pharmacol. Exp. Ther.*, **129**, 282 (1960).
15) B.B. Newbound and R. Kilpatrick, *Lancet*, **60**, 887 (1960).